

5 SIGNAL COUNTING FOR IN SITU HYBRIDIZATION

Abstract

A computer system counts fluorescently tagged nucleic acid probe signals
10 in biological specimens by determining a ratio of signals from a test probe to
signals of a reference probe. Probe signals need not be counted with reference to
cells, nuclei, or nuclear contours. Gene amplification or deletion can thus be
detected by analyzing the ratio. Successive image slices are obtained by confocal
microscopy, and the images are digitized. The digital images are transformed and
15 analyzed to combine contiguous fluorescent signal segments in successive optical
sections to identify discrete probe signals, or spots. Spots overlapping in the axial
and transverse dimensions of a three-dimensional representation of the biological
specimens can be distinguished. A graphical user interface presents various
features for consideration by a user, who can provide guidance to a computer
20 system counting the spots. Various features directed to identifying spot clusters
and autofluorescent material can increase accuracy of spot counting.